Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 8569–8571, August 1996
Immunology

A common major histocompatibility complex class II allele HLA–DQB1*0301 is present in clinical variants of pemphigoid

(PCR/HLA alleles and haplotypes/autoimmunity/pemphigoid disease)

JULIO C. DELGADO[†‡§¶], DAVID TURBAY[†‡], EDMOND J. YUNIS[†‡‖], JUAN J. YUNIS[†‡], ELIZABETH D. MORTON[†‡], KAILASH BHOL[‖**], ROBERT NORMAN[††], CHESTER A. ALPER[‡‖], ROBERT A. GOOD[‡‡], AND RAZZAQUE AHMED[‖**]

[†]Division of Immunogenetics, Dana–Farber Cancer Institute, Boston, MA 02115; [‡]Department of Pathology, Harvard Medical School, Boston, MA 02115; [‖]The Center for Blood Research, Boston, MA 02115; [§]Department of Pediatrics, Harvard Medical School, Boston, MA 02115; [††]Dermatology Health Care, Tampa, FL 33615; [‡‡]Department of Pediatrics, University of South Florida, All Children's Hospital, St. Petersburg, FL 33701; and [**]Harvard School of Dental Medicine, Boston, MA 02115

Contributed by Robert A. Good, May 15, 1996

ABSTRACT **Bullous pemphigoid (BP) is an autoimmune subepidermal blistering disease seen primarily in elderly persons. It is characterized clinically by the development of tense bullae and by the presence of an antibasement membrane antibody. In BP, the antigens involved in the autoimmunity are epidermal basement membrane peptides BPAg1 and BPAg2. We have compared high resolution typing of major histocompatibility complex class II loci (HLA–DRB1, DQB1) in 21 patients with BP, 17 with ocular cicatricial pemphigoid (OCP), and 22 with oral pemphigoid (OP) to a panel of 218 haplotypes of normal individuals. We found that the three diseases (BP, OCP, and OP) have significant association with DQB1*0301 ($P = 0.005$, $P < 0.0001$, and $P = 0.001$, respectively). The frequencies of alleles DQB1*0302, *0303, and *06, which share a specific amino acid sequence from position 71 to 77 (Thr-Arg-Ala-Glu-Leu-Val-Thr) were also increased ($P = 0.01$). We suggest that an identical major histocompatibility complex class II allele (DQB1*0301) is a common marker for enhanced susceptibility and that the same amino acid residues in positions 71–77 (DQB1*0301, -0302, -0305, -0602, -0603 alleles) are found in patients with BP, OCP, and OP. Our findings propose that the autoimmune response in the three different clinical variants of pemphigoid, involves the recognition by T cells of a class II region of DQB1, bound to a peptide from the basement membrane of conjunctiva, oral mucosa, and skin.**

Bullous pemphigoid (BP) is an autoimmune subepidermal blistering disease seen primarily in elderly persons and is characterized clinically by the development of blisters. Therapy usually involves oral corticoids and frequently immunosuppressant agents (1). There is *in vivo* deposition of immunoglobulins (Ig) and complement components at the basement membrane zone of the dermo–epidermal junction. Sera of the majority of patients contains anti-basement membrane zone autoantibodies (1).

It is postulated that the lesions in BP result from autoimmunization with IgG class autoantibodies against antigens BPAg1 and BPAg2, components of the epidermal basement membrane zone (2). Recently, it has been demonstrated that passive transfer of autoantibodies that recognize BPAg2, when injected into neonatal mice, can produce clinical BP (3).

In previous studies we have shown an association between the DQB1*0301 allele with ocular cicatricial pemphigoid (OCP) and oral pemphigoid (OP), when compared with a population of normal controls (4, 5). While in patients with pemphigus vulgaris we have found an association with HLA–DR4, DQ8 among Jewish patients, and DR6, DQ5 in non-Jewish patients (6–8).

In the present report, 21 Caucasian patients with BP were studied for major histocompatibility complex (MHC) class II DRB1, DQB1 alleles, using amplification of genomic DNA by the polymerase chain reaction and sequence-specific oligonucleotide probe hybridization. Frequencies were compared with 218 haplotypes of normal individuals.

MATERIALS AND METHODS

Twenty-one patients with BP were typed for MHC class II DRB1, DQB1 alleles. These results were compared with those obtained from analysis of 218 normal haplotypes. In addition, six patients with OCP and six with OP previously reported as HLA–DR4 were subtyped and compared with the BP patients. The diagnosis of BP was based on typical clinical features and routine histologic examination demonstrating a subepidermal vesicle. Direct immunofluorescence examination of perilesional skin demonstrated deposits of Ig and complement at the basement membrane zone (1).

DNA Isolation. DNA was separated with the Stratagene DNA isolation kit or by the salting out method with minor modifications (9).

PCR Amplifications. DNA samples were amplified by PCR for DRB1 and DQB1 loci in a 100-μl reaction mixture containing 50 mM KCl, 10 mM Tris·HCl (pH 8.3), dNTPS (each at 125 mM), 50 pmol of each primer, 1.5-2 mM of $MgCl_2$, and 2.5 units of *Taq* polymerase [AmpliTaq DNA polymerase (Perkin–Elmer/Cetus)]. The primers and conditions used in this study have been described elsewhere (10–14). Negative controls were included to detect contamination.

PCR-amplified products (5 μl) were separated by electrophoresis in TBE/2% UltraPURE Agarose (GIBCO) gel at 150 V for 30 min. (1 × TBE = 0.9 mM Tris borate/0.002 M EDTA), followed by photography of the ethidium bromide-stained DNA PCR products. Dot-blot, prehybridization, and hybridization procedures were carried out as described (12, 13).

DRB1 and DQB1 alleles were determined in the PCR-amplified products by sequence-specific oligonucleotide probe hybridization as described before (11, 12).

Statistical Analysis. The frequencies of independent haplotypes and alleles were determined by direct counting. Statistical significance of the differences in frequency of individual MHC alleles and haplotypes in the patients and members of the control population was estimated by Fisher's exact test, with the aid of INSTAT software (GraphPad, San Diego).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: BP, bullous pemphigoid; CP, cicatricial pemphigoid; OP, oral pemphigoid; OCP, ocular cicatricial pemphigoid; MHC, major histocompatibility complex.

[¶]To whom reprint requests should be addressed.

Table 1. MHC class II haplotypes in patients with BP

ID	First Haplotype		Second haplotype	
	DRB1	DQB1	DRB1	DQB1
ES	*0401	*0301	*01	*0501
AE	*0401	*0301	*03	*02
FG	*0401	*0301	*06	*0603
RA	*11	*0301	*11	*0301
JG	*11	*0301	*02	*0502
SC	*11	*0301	*02	*0502
MP	*11	*0301	*07	*02
LB	*11	*0301	*0403	*0305
FH	*11	*0301	*06	*0603
FT	*11	*0301	*07	*02
FO	*12	*0301	*02	*0603
LA	*12	*0301	*07	*02
AG	*06	*0301	*08	*0402
HF	*0402	*0301	*03	*02
BR	*0402	*0302	*03	*02
MF	*0402	*0302	*01	*0501
MM	*0402	*0302	*01	*0501
DK	*02	*0603	*02	*0603
MD	*02	*0603	*02	*0602
TC	*01	*0501	*01	*0501
ELM	*06	*05031	*06	*05031

ID, patient code. Underlined DQB1 indicate identical amino acid in positions 71–77.

RESULTS

The MHC class II alleles and haplotypes of patients with BP are shown in Table 1. The phenotype frequency of HLA–DQB1*0301 among 21 patients with BP was 66% (14 of 21). One was homozygous, and 12 were heterozygous. The frequency of DRB1*0402 was 19% (4 of 21), all heterozygous. The allele frequencies and statistical comparisons for DRB1 and DQB1 are shown in Table 2.

Comparison to the control individuals showed a statistically significant association ($P = 0.005$) (Table 2). The DRB1*0402 allele frequency was 9.52%, showing a statistically significant association with BP when compared with control individuals ($P < 0.04$) (Table 2).

Six patients with OCP and six with OP described before as DRB1*04 (5) were subtyped. None of them was found to be DRB1*0402. In that study, only one patient with OP was reported as *0402 (5).

Results of HLA typing of OCP and OP patients from a previous study (5) were compared with the control panel used in this study. A significant increase in DQB1*0301 association was found with OCP ($P < 0.0001$) and OP ($P = 0.001$). A comparison of the three subsets of pemphigoids is shown in Table 3.

DISCUSSION

Our previous studies in different forms of blistering diseases, OCP and OP, have revealed a common susceptibility marker, HLA–DQB1*0301 (4, 5). In this study, 21 patients with BP were analyzed by PCR sequence-specific oligonucleotide hybridization methods. The frequency of the allele DQB1*0301 was 35.7%, which was significantly increased when compared with control individuals ($P = 0.005$) (Table 2).

Table 2. Statistical comparison of MHC class II DRB1, DQB1 alleles in BP

Allele	BP (72)		Normal (218)		Significance (P value)
	No.	%	No.	%	BP vs. normal
DRB1					
01	5	11.9	28	12.84	NS
02	7	16.66	36	16.51	NS
03	3	7.14	25	11.46	NS
0401	3	7.14	10	4.58	NS
0402	4	9.52	5	2.29	0.04
0403	1	2.38	2	0.91	NS
0404			7	3.21	NS
0411			2	0.91	NS
11	8	19.04	21	9.63	NS
12	2	4.7	6	2.75	NS
06	5	11.9	34	15.59	NS
07	3	7.14	29	13.3	NS
08	1	2.38	5	2.29	NS
10			4	1.83	NS
DQB1					
0501	5	11.9	31	14.22	NS
0502	2	4.7	2	0.91	NS
0503	2	4.7	12	5.5	NS
0601			2	0.91	NS
0602	1	2.38	36	16.51	NS
0603	6	14.2	13	5.96	NS
0604			3	1.37	NS
0605			1	0.45	NS
02	6	14.2	8	3.66	NS
0301	15	35.71	35	16.05	0.005
0302	3	7.14	24	11	NS
0303			8	3.66	NS
0305	1	2.38			NS
0402	1	2.38	8	3.66	NS

No., the number of haplotypes; NS, not significant. Numbers in parenthesis are *n*.

Previous reports have shown that DRB1*04 was present in patients with OCP and OP (4, 5). Pemphigus vulgaris patients have statistically significant association with DRB1*0402 (Jewish) (6) and DQB1*0503 (non-Jewish) (7). We analyzed 12 patients previously reported with OCP and OP (5). None of them was found to be DRB1*0402. Interestingly, a significant association between BP patients and HLA–DRB1*0402 was detected in four patients ($P < 0.04$) (Table 2). However, it was determined that these patients had Jewish ancestry, which explains the increased association in this study.

There have been reports of associations of different DQB1 alleles with certain clinical conditions. The DQB1*0201 allele has been found to be associated with gluten-sensitive enteropathy (celiac disease) (15, 16), DQB1*0604, *0501 with dilated cardiomyopathy (17), while DQB1*0302 has been found to be associated with insulin-dependent diabetes mellitus (18). In OP and OCP patients (5), analysis of the amino acid residues showed a significant association with amino acid

Table 3. Statistical comparison of HLA–DBQ1* alleles in BP, OCP, and OP

Allele	BP (42)		OCP (34)		OP (44)		Normal (218)		Significance (P value)		
	No.	%	No.	%	No.	%	No.	%	BP vs. normal	OCP vs. normal	OP vs. normal
DQB1											
0301	15	35.7	18	52.9	17	38.6	35	16.05	0.005	0.0001	0.001

No., the number of independent haplotypes; NS, not significant. Numbers in parenthesis are *n*.

positions 71–77 of the DQB1 gene in the second exon. We found that 19 of 21 BP patients (90%) carried identical amino acid residues in positions 71–77 compared with 89 of 130 control individuals ($P = 0.01$) (Table 1, underlined haplotypes). An association of the DQB1*0301, -0302, -0303, and -0602 alleles, which share identical amino acid residues in positions 71–77 and the presence of anti-phospholipid antibodies in several different clinical presentations of systemic lupus erythematosus has been reported (19).

Our findings suggest that either an identical MHC class II marker for susceptibility (DQB1*0301) is present in three clinical subsets of pemphigoid or the susceptibility is conferred by amino acid residues in positions 71–77 as these were observed in 90% of patients with BP. This would suggest two possible explanations. The first, that the same peptide sequence within different pemphigoid antigens is recognized by different alleles (DQB1*0301, -0302, -0305, -0602, and 0603). The second, that there are different epitopes within the three variants of pemphigoid studied and that the amino acid residues in position 71–77 of DQB1 bind them equally for presentation to the helper T cells. Studies involving synthetic peptides and T cell responses in BP patients will provide an insight and an understanding of this phenomenon.

This work was supported by Grants EY 08379 and DE 09978 to A.R.A. and HL 29583 to E.J.Y. and C.A.A. from the National Institutes of Health and in part by the Rubenstein Foundation. The authors thank Tazim Verjee for manuscript preparation and editing.

1. Ahmed, A. R. & Hameed, A. (1993) *Clin. Dermatol.* **11,** 47–52.
2. Giudice, G. J., Squiquerra, H. L., Elias, P. M. & Diaz, L. A. (1991) *J. Clin. Invest.* **87,** 734–738.
3. Liu, Z., Giudice, G. J., Swartz, S. J., Fairley, J. A., Till, G. O., Troy, J. L. & Diaz, L. A. (1995) *J. Clin. Invest.* **95,** 1539–1544.
4. Ahmed, A. R., Foster, S., Zaltas, M., Notani, G., Awdeh, Z., Alper, C. A. & Yunis, E. J. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 11579–11582.
5. Yunis, J. J., Mobini, N., Yunis, E. J., Alper, C. A., Deulofeut, R., Rodriguez, A., Foster, C. S., Marcus-Bagley, D., Good, R. A. & Ahmed, A. R. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 7747–7751.
6. Ahmed, A. R., Yunis, E. J., Khatri, K., Wagner, R., Notani, Z., Awdeh, Z. & Alper, C. A. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7658–7662.
7. Ahmed, A. R., Wagner, R., Khatri, K., Notani, G., Awdeh, Z., Alper, C. A. & Yunis, E. J. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 5056–5060.
8. Ahmed, A. R., Mohimen, A., Yunis, E. J., Mirza, N. M., Kumar, V., Beutner, E. H. & Alper, C. A. (1993) *J. Exp. Med.* **177,** 419–424.
9. Miller, S. A., Dykes, D. D. & Polesky, H. F. (1988) *Nucleic Acids Res.* **16,** 1215-.
10. Yunis, J. J., Delgado, M. B., Lee-Lewandroski, E., Yunis, E. J. & Bing, D. H. (1992) *Tissue Antigens* **40,** 116–123.
11. Salazar, M., Yunis, J. J., Delgado, M. B., Bing, D. & Yunis, E. J. (1992) *Tissue Antigens* **40,** 116–123.
12. Yunis, J. J., Salazar, M., Delgado, M. B., Alper, C. A., Bing, D. H. & Yunis, E. J. (1993) *Tissue Antigens* **41,** 37–41.
13. Kimura, A. & Sasazuki, T. (1991) in *HLA 1991: Proceedings of the Eleventh International Histocompatibility Workshop and Conference*, eds. Tsuji, K., Aizawa, M. & Sasazuki, T. (Oxford Univ. Press, Oxford), pp. 397–419.
14. Marsh, S. G. (1992) *Tissue Antigens* **40,** 229–243.
15. Ahmed, A. R., Yunis, J. J., Marcus-Bagley, D., Yunis, E. J., Salazar, M., Katz, A. J., Awdeh, Z. & Alper, C. A. (1993) *J. Exp. Med.* **178,** 2067–2075.
16. Sollid, L. M. & Thorsby, E. (1993) *Gastroenterology* **105,** 910–922.
17. Limas, C. J., Limas, C., Goldenberg, I. F. & Blair, R. (1995) *Am. Heart J.* **129,** 1141–1144.
18. Todd, J. A., Bell, J. J. & McDevitt, H.O. (1987) *Nature (London)* **329,** 559–563.
19. Arnett, F. C., Olsen, M. L., Anderson, K. L. & Reveille, J. D. (1991) *J. Clin. Invest.* **87,** 1490–1495.